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阿魏酸交联处理对明胶性质的影响*

井乐刚[1] 赵新淮[2]

1. 哈尔滨师范大学生命科学与技术学院 哈尔滨 150025
2. 东北农业大学乳品科学教育部重点实验室 哈尔滨 150030

摘 要 采用恒重法测定了交联明胶在25℃的水吸附等温曲线, 分别用质构仪和乌氏黏度计测定了不同浓度阿魏酸交联明胶溶液的凝胶强度和黏度, 评价了阿魏酸交联处理对明胶的水吸附性、胶凝性和黏性的影响。结果表明: 在水分活度相同的条件下交联明胶的平衡含水量在水分活度较低时略低于明胶, 而在水分活度较高时则略高于明胶; 可以用GAB模型很好地拟合水吸附等温数据; 交联明胶溶液形成凝胶的临界质量浓度为0.7% g/mL, 与明胶接近; 在临界浓度以上交联明胶的凝胶强度随着溶液浓度的增加而提高, 但是明显地比相同浓度下明胶的强度低; 交联明胶的特性黏数(354.38 mL/g)比明胶的(85.80 mL/g)高。

关键词 有机高分子材料, 交联明胶, 阿魏酸, 水吸附等温曲线, 凝胶强度, 黏度

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Effects of Cross-linking Treatment of Ferulic Acid on Properties of Gelatin

JING Legang[1**] ZHAO Xinhuai[2]

1. College of Life Science and Technology, Harbin Normal University, Harbin 150025, China

2. Key Lab of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin 150030, China

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**To whom correspondence should be addressed, Tel: (0451)88060576, E-mail: jlgachxy@126.com

ABSTRACT Cross-linked gelatin has been prepared using ferulic acid as cross-linking agent in a previous article. The moisture sorption isotherm of the cross-linked gelatin was determined gravimetrically at 25℃. The gel strength and the viscosity of solutions with different concentrations of cross-linked gelatin were determined by texture analyzer and Ubbelohde viscosimeter, respectively. The results show that for the salt saturated solutions with the same water activity, the equilibrium moisture content of the cross-linked gelatin is slightly lower than that of the non cross-linked gelatin in the solutions with low water activity, but the former is slightly higher than the latter when the water activity is high. The moisture sorption isotherm data were mathematically fitted to the GAB model. The critical concentration of the cross-linked gelatin solution to form gel is 0.7% g/mL, which was similar to that of the non cross-linked gelatin solution. Above the critical concentration, the gel strength of the cross-linked gelatin increases with the increase of its concentration, but it is obviously lower than that of the non cross-linked gelatin for the same concentration. The intrinsic viscosity of the cross-linked gelatin is 354.38 mL/g, but that of the non cross-linked gelatin is 85.80 mL/g.

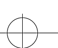
KEY WORDS organic polymer materials, cross-linked gelatin, ferulic acid, moisture sorption isotherm, gel strength, viscosity

明胶是一种将动物的皮、筋、骨胳等组织中的胶原部分水解而得到的蛋白质。作为一种重要的高分子材料, 明胶具有无毒、价格低廉、成膜性和生物相容性好等特点, 得到了广泛应用[1-7]。但是明胶易吸湿、易降解、温度高时易溶于水, 使其应用受到很大限制, 特别是在医药领域。加入交联剂使明胶改性制备交联明胶, 可改善其功能性质[8-13]。常用作

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本文联系人: 井乐刚, 教授



明胶交联剂的醛类物质, 如甲醛[14]、戊二醛[15-17]、乙二醛[14, 18-20]、乙醇醛[18]等, 虽能与蛋白质迅速结合, 但有较大毒性。一些酶类如转谷氨酰胺酶[21-26], 以及天然交联剂京尼平(genipin)[27-29]等, 也曾用作明胶的交联剂, 但是成本较高。植物酚酸来源广泛、安全无毒, 近年来国内外学者将其作为明胶等蛋白质的交联剂进行过一些研究。Strauss 等[30]以咖啡酸、绿原酸、阿魏酸等将酚酸作为交联剂使明胶产生交联, 提出了酚酸与蛋白质交联的反应机制。Cao 等[31]以阿魏酸和单宁酸为交联剂制备交联明胶膜, 研究了交联剂浓度和成膜溶液pH值对明胶膜的机械特性、膨胀性和水蒸气透过率的影响。Jiang 等[32]研究了阿魏酸交联改性对罗非鱼皮明胶抗氧化能力、水蒸气透过率和水溶性的影响。在前期研究中, 本文作者曾以阿魏酸为交联剂, 在有过氧化氢的条件下通过辣根过氧化物酶催化而制得交联明胶, 并考察了温度、pH值、交联剂浓度等因素对交联度的影响[8]。本文以阿魏酸为交联剂制备交联明胶, 并表征其性能。

1 实验方法

1.1 交联明胶的制备

实验用原料: 明胶(gelatin)(B 型), 食品级; 辣根过氧化物酶(horseradish peroxidase)(RZ 2.67, 活力 220 U/mg); 阿魏酸(ferulic acid), 分析纯; 过氧化氢(hydrogen peroxide), 分析纯; 去离子水(deionized water); 氢氧化钠(sodium hydroxide), 分析纯; 无水乙醇(anhydrous ethanol), 分析纯。

按照文献[8]优化出的交联条件制备交联明胶。将适量的明胶置于250 mL锥形瓶中, 加入去离子水后在40℃水浴加热使其溶解, 配制成9% g/mL的明胶溶液; 加入浓度为200 mmol/L 的阿魏酸贮备液使阿魏酸浓度为40 mmol/L, 然后加入浓度为1 mol/L 的 H_2O_2 溶液使 H_2O_2 和阿魏酸的摩尔比为1∶1。在锥形瓶中混合均匀后，用0.05 mol/L NaOH溶液将此体系的pH值调到8.0。然后加入0.2 mg/mL 的辣根过氧化物酶溶液, 使酶活力为0.2 U/mL。将此反应体系放入水浴振荡器中在40℃反应24 h, 取出后冷冻干燥, 粉碎后过100目筛。将产物用无水乙醇反复洗涤以除去残留的阿魏酸, 再将其在80℃烘干以除去乙醇, 得到交联度为10%的交联明胶。

1.2 交联明胶性能表征

紫外光谱: 用紫外可见分光光度计(UV-2401PC)分别测定明胶(0.25 mg/mL)、阿魏酸(0.01 mg/mL)和交联明胶(0.25 mg/mL)的紫外吸收光谱, 以蒸馏水为空白进行扫描, 扫描波长范围为190−400 nm。

红外光谱: 用傅立叶变换红外光谱仪(FTIR)(AVATAR360) 分析明胶和交联明胶的红外光谱。用KBr压片制样法, 分辨率: 4 cm^{-1}, 扫描次数: 32 次; 测试范围: 400−4000 cm^{-1}。

水吸附等温曲线: 用恒重法测定明胶和交联明胶在25℃的水吸附等温曲线[14]。分别准确称取粉碎、干燥后的明胶和交联明胶样品1.0−1.5 g, 置于已经恒重的称量瓶中, 并将此样品瓶放入密封良好的干燥器上部, 干燥器下部放置饱和盐溶液。饱和盐溶液的水分活度 a_w 列于表1。待样品恒重后取出称其质量, 计算其水分含量 m; 再用水分活度仪(Aqualab Series 3TE)测定样品的水分活度 a_w。使用Matlab R2009b 软件将水分含量和水分活度的数据用GAB (Guggenheim-Anderson-de Boer)模型进行拟合, 绘制交联明胶和明胶在25℃的水吸附等温曲线, 并给出相应的参数。GAB模型的表达式为

$$m = \frac{ckm_0a_w}{(1-ka_w)(1-ka_w+cka_w)}$$

式中 m 为样品的平衡含水量, a_w 为样品的水分活度, c 和 k 为常数, m_0 为单分子层水含量。

凝胶强度: 在一定量的粉碎、干燥后的明胶和交联明胶中加入一定量的蒸馏水, 在室温下浸泡30 min。然后进行40℃水浴加热使其完全溶解, 分别配制成0.1%、0.2%、0.3%、0.4%、0.5%、0.6%、0.7%、0.8%、0.9%、1.0% g/mL 的溶液。再各取溶液15 mL置于25 mL烧杯中, 在10℃胶凝16−18 h。取出凝胶后, 用质构仪(TA-XT Plus)测定凝胶强度。所用探头为SMS P/0.5, 下降速率为1 mm/s, 下压距离为4 mm。凝胶被压缩4 mm所需施加的最大应力为

表1 饱和盐溶液的水分活度

Table 1 Water activity of saturated salt solutions at 25℃

Saturated salt solution	a_w
CH_3COOK	0.224
$MgCl_2$	0.330
K_2CO_3	0.432
$Mg(NO_3)_2$	0.528
NaCl	0.752
KCl	0.843
KNO_3	0.936

凝胶强度。

黏度: 将粉碎、干燥后的明胶配制成质量浓度分别为0.001、0.002、0.003、0.004、0.005、0.006、0.008 g/mL的溶液, 将同样处理的交联明胶配制成质量浓度分别为0.0003、0.0004、0.0005、0.0006、0.0008、0.0010、0.0012、0.0016 g/mL的溶液。在40℃(±0.1℃)恒温水浴中, 用乌氏黏度计测量黏度。液体流过黏度计毛细管的时间通过精度为0.01 s的电子秒表计时。每个数据点至少进行5次重复试验, 每两次测量的差值不大于0.2 s, 取5次测量结果的平均值。

2 结果和讨论

2.1 紫外光谱

图1给出了明胶、阿魏酸和交联明胶的紫外吸收光谱。明胶只在215.5 nm有一个吸收峰, 由明胶中C=O的π→π*跃迁产生。阿魏酸有3个吸收峰, 分别在311.5 nm、288.5 nm和215.5 nm, 都产生于阿魏酸分子中苯环的π→π*跃迁。交联明胶有2个吸收峰, 分别在285.5 nm和213.5 nm。交联明胶的紫外吸收光谱在285.5 nm出现一个新的吸收峰, 将交联明胶用无水乙醇反复洗涤后此峰依然保留。据此可以判断, 交联明胶的吸收光谱不是明胶与阿魏酸吸收光谱的叠加, 而是明胶与阿魏酸产生交联的结果。

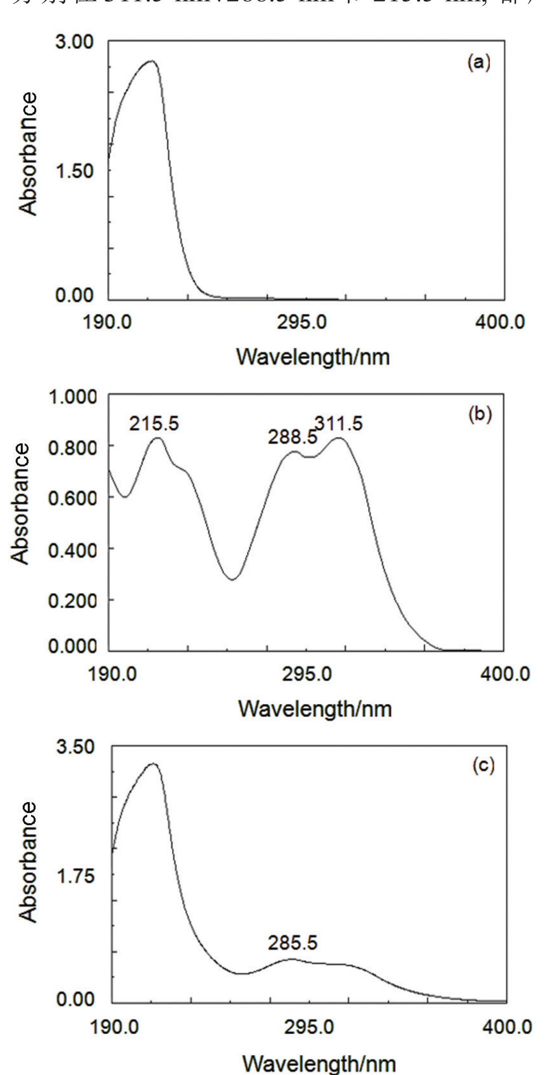


图1 明胶、阿魏酸和交联明胶的紫外吸收光谱
Fig.1 UV absorption spectra of gelatin (a), ferulic acid (b) and cross-linked gelatin (c)

2.2 红外光谱

图2给出了阿魏酸交联处理前后明胶的红外光谱。在交联前的明胶谱图中, 1654.06、1549.88、1241.10 cm^{-1}处的吸收峰分别归属于明胶酰胺键中的C=O伸缩振动(酰胺Ⅰ)、N—H弯曲振动(酰胺Ⅱ)、C—N伸缩振动(酰胺III) (图2曲线b), 这些峰的位置和其他学者[33-35]报道的相似。图2曲线b中3330.65 cm^{-1}处宽而强的吸收峰, 可归属于明胶中的N—H伸缩振动及O—H伸缩振动。交联处理后, 阿魏酸分子中—OH的引入使O—H伸缩振动加强, 因此曲线a中在3343.94 cm^{-1}处出现类似的吸收峰。曲线b中2953.79 cm^{-1}处吸收峰, 归属于C—H伸缩振动。交联处理后, 由于阿魏酸分子中=C—H伸缩振动, 比明胶中原有C—H伸缩振动的频率高, 因此曲线a中在2966.74 cm^{-1}处出现吸收峰[36]。本文的交联明胶的交联度(10%)较低, 对原料明胶的分子结构改变较小, 因此交联处理前后的谱图差异不大。

2.3 水吸附等温曲线

图3给出了明胶和交联明胶用GAB模型拟合的水吸附等温曲线, 拟合效果及各参数值列于表2。可以看出, 在水分活度较低(在0.85以下)时, 明胶的平衡含水量略高于交联明胶, 说明明胶的水吸附能力比交联明胶略高。其原因是, 明胶交联后在

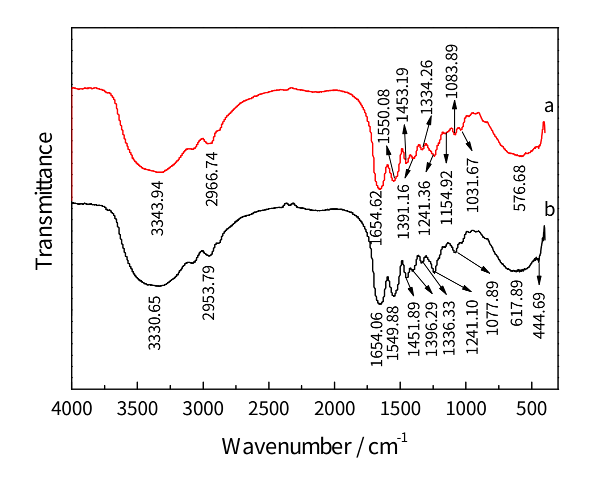
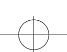


图2 交联明胶和明胶的红外光谱图
Fig.2 FTIR spectra of cross-linked gelatin (a) and gelatin (b)



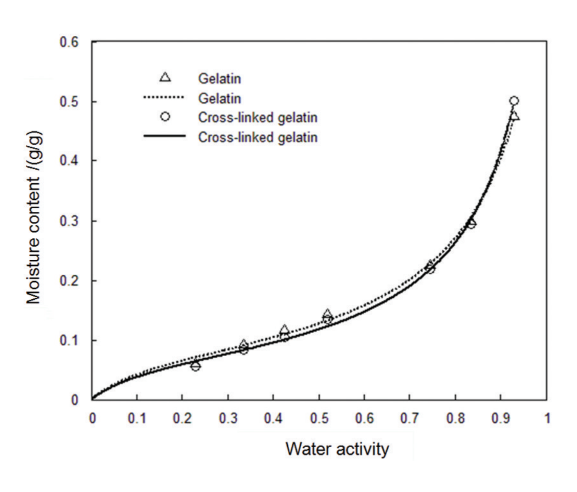


图3 用GAB方程拟合的明胶与交联明胶25℃时的水吸附等温曲线

Fig.3 Moisture sorption isotherms for gelatin and cross-linked gelatin at 25℃ fitted by the GAB equation

分子中引进了疏水性的芳香环, 并减少了亲水性的氨基。这使氢键数目减少, 从而降低了吸水性。但是, 由于交联度较低, 引进的阿魏酸残基不多, 因此明胶与交联明胶的水吸附能力差别不大。当水分活度较高(在0.85以上)时, 交联明胶的平衡含水量则高于明胶, 可能是一些亲水性的活性位点随着交联明胶的膨胀而暴露出来造成的。明胶和交联明胶吸附等温曲线的上述特征, 与前人[14, 22, 37]的研究结果一致。

2.4 凝胶的强度

图4给出了不同浓度的明胶和交联明胶溶液所形成凝胶的凝胶强度。当明胶和交联明胶溶液质量浓度均为0.1%-0.5% g/mL时, 二者的凝胶强度相等且不随浓度而变化。其原因是, 此时的溶液浓度都很低, 还没有形成凝胶的网络结构, 或者即使形成网络结构也不充分, 对探头的阻力主要由溶胶产生。当明胶溶液浓度达到0.6%、交联明胶溶液浓度达到0.7%时, 凝胶强度都突然增大, 说明此时凝胶网络结构开始形成。然后, 随着溶液浓度的增加, 明胶和交联明胶的凝胶强度都逐渐增大。因为分子相互缠结形成的凝胶网络结构逐渐致密, 网络的结点数目逐渐增多, 分子间作用力逐渐增强。在胶凝点之后,

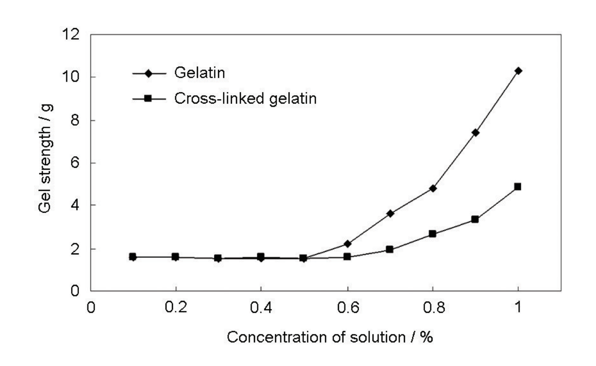


图4 明胶和交联明胶的凝胶强度

Fig.4 Gel strengths of gelatin and cross-linked gelatin

对于相同浓度溶液形成的凝胶交联明胶的强度值低于明胶, 而且随着溶液浓度的增加这种变化还有增大的趋势。凝胶网络结构主要通过分子间氢键形成, 同时也受静电斥力和疏水相互作用的影响。交联后凝胶强度减小, 可能是蛋白质侧链之间通过极性基团与阿魏酸交联作用导致形成氢键的能力减弱[38]。在溶液浓度相同时交联明胶溶液的pH值比明胶溶液高(例如, 溶液浓度均为1.0%时, 明胶溶液的pH值为6.5, 而交联明胶则为8.4), 偏离等电点较远, 分子间的静电排斥作用较强, 因而形成较弱的凝胶; 随着二者溶液浓度的增大, 交联明胶溶液的pH值偏离等电点变远, 分子间静电斥力变大。虽然凝胶强度也逐渐增加, 但增加幅度没有明胶大。明胶和交联明胶凝胶强度的上述变化规律, 与邵士风等[38]等的研究结果一致。

2.5 黏度

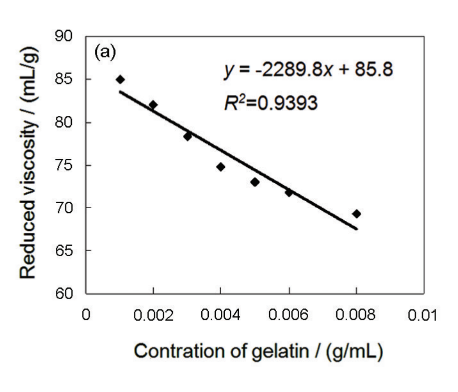
图5给出了明胶和交联明胶溶液的比浓黏度。可以看出, 随着溶液浓度的增加, 明胶和交联明胶溶液的比浓黏度都降低。但是两条直线的斜率表明, 交联明胶溶液的比浓黏度降低速率更快。同时, 按照图中的两个回归方程计算可知, 在浓度相同时交联明胶溶液的黏度比明胶的大[39]。图中的线性方程显示, 明胶溶液的特性黏数为85.80 mL/g, 而交联明胶则为354.38 mL/g。由于测定二者溶液黏度时的温度及所用溶剂均相同, 因此可初步判断交联明胶的相对分子质量比明胶大。这显然是明胶交

表2 明胶与交联明胶水吸附GAB方程回归分析结果

Table 2 Regression results of moisture sorption for gelatin and cross-linked gelatin by GAB equation

Sample	m_0 /(g/g)	c	k	R^2	SSE	RMSE
Gelatin	0.07936	9.136	0.8981	0.9971	0.0003694	0.009610
Cross-linked gelatin	0.07116	8.909	0.9245	0.9979	0.0003126	0.008841

Note: R^2, coefficient of determination; SSE, sum of squares for error; RMSE, root mean square error



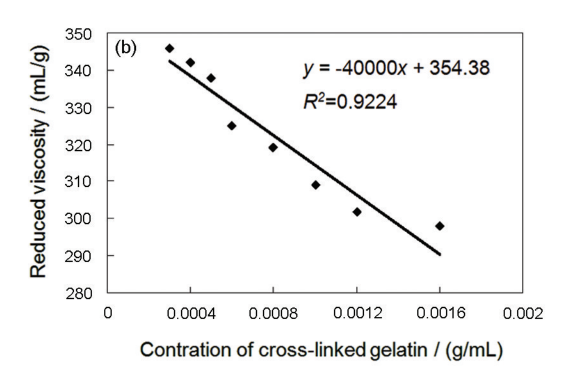


图5 明胶和交联明胶的比浓黏度

Fig.5 Reduced viscosity of gelatin (a) and cross-linked gelatin (b)

联后的必然结果, 也为明胶的交联提供了进一步的证据。

3 结　论

用阿魏酸交联处理的明胶, 在相对湿度较低时水吸附能力略低于明胶, 而在相对湿度较高时则比明胶高。交联明胶溶液形成凝胶的临界质量浓度为0.7% g/mL, 与明胶接近; 在临界浓度以上, 交联明胶的凝胶强度随溶液浓度的增加而增加, 但是明显低于相同浓度下的明胶。交联明胶的特性黏数高于明胶, 可初步判断交联明胶的相对分子质量比明胶大。

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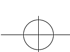
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